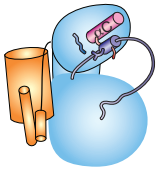
CELL DEATH

Jamming the switch

Proc. Natl. Acad. Sci. USA
doi:10.1073/pnas.1408987111

PNAS



The induction of necroptosis requires receptor interacting protein kinase-3 (RIPK3)-mediated phosphorylation of the activation loop in the pseudokinase mixed lineage kinase domain-like (MLKL). MLKL phosphorylation has been proposed to lead to a conformational change in the pseudokinase domain that promotes MLKL oligomerization and plasma membrane localization, thereby activating downstream cell death effectors. To determine the MLKL domains required for cell death, Hildebrand *et al.* expressed MLKL fragments in *mlkl*-deficient fibroblasts under control and necroptosis-induced conditions. Expression of the pseudokinase domain was sufficient to block necroptosis, whereas expression of the *N*-terminal four-helix bundle (4HB) domain promoted constitutive necroptosis. Consistent with the importance of the 4HB domain, alanine scanning mutagenesis revealed two clusters in 4HB critical for the function of MLKL; mutations in cluster 1 prevented MLKL oligomerization and membrane localization, whereas mutations in cluster 2 exhibited normal membrane localization but were unable to initiate cell death. The authors proposed that 4HB is normally constrained by the pseudokinase domain and that RIPK3-induced conformational changes in the pseudokinase domain may free the 4HB domain to promote cell death. To test their model, the authors identified a small molecule that binds and disrupts the pseudokinase domain. Treatment with this small molecule prevented membrane localization and necroptotic cell death, suggesting that coordination between the pseudokinase and 4HB domains may be essential to control entry into necroptosis. *GM*

CELL WALLS

Cellulose snakes along

J. Biol. Chem. doi:10.1074/jbc.M114.607192

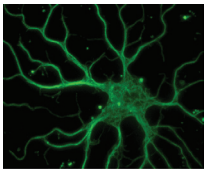
Cellulose polymers found in plant cell walls are synthesized by cellulose synthase complexes (CESA), but the mechanism for conversion of individual polymers into microfibrils remains unclear. COBRA is coexpressed with CESA, and mutations of COBRA are linked to changes in cellulose, but its specific function is unknown. Sorek *et al.* now connect these aspects in their study of COBRA localization and impacts on cellulose assembly. The authors first track COBRA in elongating root cells, confirming the protein is found at the plasma membrane, as expected given its previously identified GPI anchor. COBRA was less abundant than CESA and moved through the cell more slowly, suggesting that the proteins were not directly coupled; however, COBRA was strongly depleted by a cellulose synthase inhibitor, supporting a functional link. Similarly, tracking CESA in wild-type cells and a COBRA knockout indicated that COBRA slows down but does not otherwise affect movement of CESA. *In vitro*, COBRA binds both individual glucan chains and crystalline cellulose but shows a preference for individual chains in a direct competition assay. Analysis of cell walls by solid state NMR showed that cellulose is decreased in knockout cells and that the remaining cellulose is less crystalline and contains a larger number of shorter glucan chains. The combined results lead the authors to propose that COBRA functions in proximity to, but not associated with, CESA to align glucan chains and create cellulose microfibrils. *CG*

TRANSPORTERS

A metal movement disorder

J. Neurosci. **34**, 14079–14095 (2014)

SOMSHUVRA MUKHOPADHYAY



Exposure to elevated levels of manganese (Mn) or a defect in Mn excretion by the liver can lead to an irreversible parkinsonian-like syndrome that shares some but not all clinical and pathological features with Parkinson's disease. Recent work has identified a gene linked to a familial form of Mn-induced Parkinsonism, *SLC30A10*, but its cellular function and disease mechanism have been unknown. Leyva-Illades *et al.* set out to identify the gene product and began by demonstrating its localization at the cell membrane of HeLa cells. Five disease-linked SLC30A10 mutants, including Δ105–107 and L89P, were mislocalized to the endoplasmic reticulum (ER), where they are tagged for proteasomal degradation. The L89P mutant was also ER localized in two different cell types tested in whole *Caenorhabditis elegans* worms, as was Δ105–107 in a rat neuronal cell line and in mouse primary midbrain neurons. Given clues from its sequence, the authors next tested the metal-transporting ability of SLC30A10. A direct assay of metal transport and pulse chase experiments suggested that SLC30A10, but not the mutants, acts as an efflux transporter to lower cellular Mn levels. SLC30A10 expression protected HeLa cells as well as neurons and worms against Mn-induced toxicity, whereas the Δ105–107 or L89P mutations or *SLC30A10* knockdown did not. These results suggest that the Parkinsonism caused by mutation in *SLC30A10* is a result of a block in Mn efflux from affected cells. *MB*

DRUG DISCOVERY

Minimalist synthesis

Nat. Chem. **6**, 877–884 (2014)

Historically, drug discovery has been driven by screening the products of microbial fermentation for bioactive nonribosomal peptides. Although such serendipity served chemists well in the past, the future lies in more directed approaches. Huang *et al.* describe how to 'grow' desired synthetic peptides, using off-the-shelf building blocks and amide-mediated ligation. The process uses three types of components—initiators, elongation monomers and terminators—that are mixed in different ratios in small volumes of aqueous, reagent-free solutions. The peptide sequence can be regulated by altering the order of elongation monomer addition. The approach offers several benefits: the peptides can be tested for bioactivity without further purification, and the ease of synthesis eliminates the need to store the products as they can be readily regenerated. The combinatorial nature of the approach makes accessible a vast space of potential compounds. For example, using just 23 components that approximate parts of a known inhibitor of the hepatitis C virus protease, which is recognized as a difficult target owing to its lack of a well-defined active site, the authors generated 6,000 lead molecules. From these, a compound with a half-maximum inhibitory concentration of 1 μM was identified. This approach may have more general applications, however; molecules that have no biological activity but are more relevant to materials science or chemical catalysis might also be constructed by the appropriate choice of elongation monomers. *AKE*